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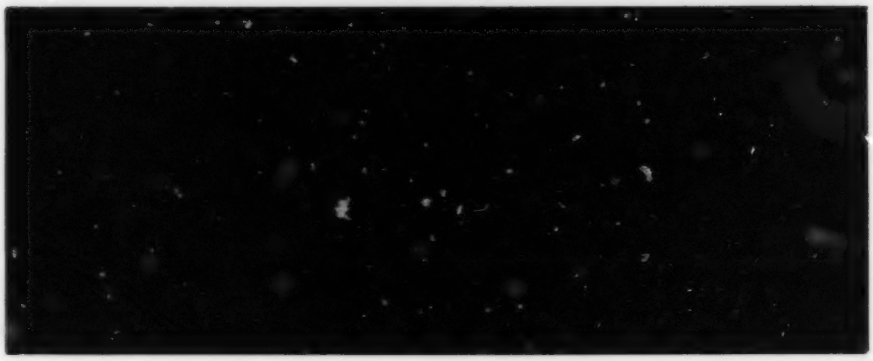

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Final Report

2.4-34

Studies on Immunity to Ureaplasma Diversum Infections in Beef Heifers

Conducted by
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Funded by the Agriculture Development Fund

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FINAL REPORT ON ADF/ERDA PROJECT 2.4-34 "STUDIES ON IMMUNITY TO UREAPLASMA DIVERSUM INFECTIONS IN BEEF HEIFERS"

(a) Executive Summary

(1) Abstract

The objectives were to determine: (a) whether genital ureaplasma infection stimulated antibodies associated with clearance of organisms and clinical recovery; and (b) the feasibility of vaccination.

Experimental infections induced a genital antibody response which was associated with recovery from vaginitis, but the organisms remained. Subcutaneously-inoculated, mildly adjuvated vaccines stimulated high serum-antibody/low vaginal antibody that was non-protective. Subcutaneously or intravaginally inoculated, adjuvated vaccines induced high serum and vaginal antibody that neither protected against challenge nor cleared the ureaplasmas.

Therefore, although vaccination was promising, identification of the antibody-inducing components (antigens) of the ureaplasmas was considered essential to the development of better vaccines.

(2) Summary and Impact

In a preliminary experiment, both ureaplasma-vaccinated (subcutaneously) and placebo-vaccinated heifers developed granular vaginitis after challenge with viable ureaplasmas; the vaginitis cleared-up in both groups during the 35-day-observation period but the organisms were consistently cultured (not cleared). Post-vaccinaton, only the vaccinates developed specific antibodies in their serum and vaginal mucus (VM), but post-challenge both groups developed increased levels of antibody, particularly in VM. The untreated control group remained free of ureaplasmas and antibodies. Therefore, subcutaneous vaccination induced serum antibody but minimal VM antibody that did not prevent infection or clear the ureaplasmas.

A second trial of 20 heifers in five groups (4 principals, 1 control) looked at the effect of intravaginal (3 groups) versus subcutaneous (1 group) vaccination with two types of vaccines (WC-whole cells; CM-cell membranes) with or without adjuvant (complete Freund's) on the clinical and immunological response to intravaginal challenge. Subcutaneous vaccination (WC vaccine + adjuvant) induced high antibody (principally of immunoglobulin types IgG_1 and IgG_2 with minimal IgM and no IgA)

in serum and VM. Vaginal vaccination with either vaccine + adjuvant stimulated low IgA in VM only and high IgG_1/IgG_2 in both samples.

After challenge (high dose), vaginitis developed in all vaccinated and control heifers. Histological examination of vaginal biopsies revealed an accumulation of lymphocytes and neutrophils beneath the inflamed epithelium. The post-challenge infection stimulated a markedly increased IgA antibody response in the VM as compared to post-vaccination levels. As in the preliminary experiment, the vaginitis gradually subsided but all heifers remained vaginal carriers for the 50-day-observation period. The post-challenge, cell-mediated immunity of these heifers was studied by response to intradermally-injected ureasplasma extracts and their lymphocytes' response to this material. The three groups vaccinated subcutaneously or intravaginally with either vaccine and adjuvant developed significantly greater skin reactivity (thick) than the control group or the group vaccinated intravaginally with non-adjuvated CM vaccine. Peripheral blood lymphocytes from heifers in the same three groups also had significantly greater lymphocytic reactivity than the control or the non-adjuvated vaccine group.

Our general conclusions were: (a) both vaccination (adjuvated vaccines) and ureasplasma infection stimulated the antibody and cell-mediated components of the immune response; (b) infection stimulated higher levels of IgA in vaginal mucus than did vaccination and this may explain why vaccination did not prevent infection or rapidly clear the ureaplasma.
In terms of agricultural impact we learned than ureaplasma vaginitis stimulated a specific immune response (both antibody and lymphocytic) that was quantitated. Vaccination with adjuvated vaccines also stimulated a good immune response but was of inferior quality (compared to infection) in that it did not prevent infection from a high-challenge dose or reduce/eliminate carriage of ureaplasmas. Further studies with this system appear to be justified, the two major objectives being (a) to identify the protection-inducing proteins in ureaplasmas, and (b) to determine if the IgA-type antibody in vagiral mucus is a major factor in the prevention of ureasplasm infection.

(b) Technical Report

Abstract

In a preliminary study, subcutaneous vaccination of heifers with mildly adjuvated, killed U. diversum stimulated specifc IgG_1 and IgG_2 antibody responses in serum and vaginal mucus (VM) but there was not an IgM or IgA response. Subsequent challenge with viable ureaplasmas produced a granular vaginitis in both vaccinated and placebo-vaccinated heifers; there was a corresponding marked increased in IgA antibody in VM of both

groups as well as a slight increase in IgG_1 and IgG_2 levels in serum. It was concluded that subcutaneous vaccination stimulated IgG antibodies in serum and VM but failed to stimulate local production of IgA (as was seen in vaginal infections with ureaplasmas) and consequently might explain the lack of protection from vaccination.

A second study of the response to subcutaneous or intravaginal vaccination with adjuvated, ureaplasma whole cell (WC) or cell membrane (CM) vaccines in heifers that were subsequently challenged intravaginally shed more light on antibody and cellular reactions. Subcutaneous vaccination with adjuvated CM vaccine elicited high levels of IgG's in serum and VM, minimal levels of IgM in both but no detectable IgA. Intravaginal vaccination, with either vaccine plus adjuvant, stimulated high levels of IgG's in serum and VM, minimal levels of IgM in both fluids and low levels of IgA only in VM. Intravaginal vaccination with the CM vaccine, without adjuvant, stimulated minimal levels of IgG's and IgM in both serum and VM but no IgA. Subsequent challenge intravaginally, resulted in granular vaginitis in all vaccinated and control heifers, with all animals remaining culture-positive for 50 days. The challenge infection stimulated a marked increase in IgA in VM in all adjuvant-vaccinated groups as compared to post-vaccination levels. After challenge, all heifers vaccinated with adjuvated vaccine had a significantly increased skin reactivity to ureasplasma antigens when compared with the non-adjuvated vaccine group or the control, non-vaccinated group. Similarly, peripheral blood lymphocytes from the heifers vaccinated with adjuvated vaccine had a significantly increased reactivty to ureasplasma antigens in comparison with the non-adjuvated vaccine group or the control group. The lack of protection from vaccination against challenge may be due to poor stimulation of IgA in VM, too high a challenge dose or a combination of these and unknown factors.

Background and Objectives

Ureasplasmas cause a granular vulvo-vaginitis which is associated with infertility in the cow, sporadic abortions and rarely seminovesiculitis in bulls. Reproductive problems in cattle are very important and, if caused by microorganisms there is a potential for control by vaccination or antimicrobial therapy. It is known that recovery from granular vaginitis eventually occurs (weeks to months) but little is known about the immune response to ureasplasma or the potential value of vaccination. Consequently, the ultimate objective of this project was to generally characterise the humoral (antibody) immune response of heifers to experimental infection of the genital tract with U. diversum and to see how this could be modified with vaccination by various routes. In addition to study of serum antibody the question of the importance of secretory or local antibody in vaginal mucus needed to be answered since the infection is localized to the genital tract.

The question of the role of cells such as lymphocytes in the immune response was considered to be of secondary importance but also needed to be examined.

Experimental Methods

It was necessary to select non-pregnant heifers that did not have granular vaginitis and were culturally negative for ureaplasmas so heifers were purchased subject to these requirements. This resulted in a rejection rate of up to 50%.

Microbiological tests included culturing of vaginal swabs and tissues on ureaplasma agar/broth media, growing large quantities of U. diversum in broth for preparation of vaccines, as well as for ELISA antigens needed for quantitation of antibodies. For determination of levels and types of antibody by ELISA (enzyme-linked immunosorbent assay), microtitre plates were coated with 100 ul of ureaplasma antigen and then reacted sequentially with the heifers' sera or VM extracts, appropriate antiglobulins, horse-radish peroxidase conjugate and the enzyme color indicator system prior to Titertek spectrophotometric reading. For the skin hypersensitivity tests on the heifers post-challenge two antigens were used, namely sonicated ureaplasma and sodium-azide killed whole cells. At 36 days after challenge, the antigens (0.1 ml amounts) and a broth control were injected intradermally at 3 surgically prepared sites in all heifers. The skin thickness at these sites was measured by a vernier metric caliper prior to injection and at 4, 24, 48, and 72 hours post-injection.

Also at 56 days post-challenge in order to measure the reactivity of peripheral blood lymphocytes (PBL) to ureaplasma antigen, 50 ml of blood in heparinized tubes was collected from each heifer. The PBL were separated by standard Ficoll-paque method and set up (with ureaplasma antigen added) in quadruplicate in cell culture in microtitre plates for 120 hours at which time they were labelled with 34-thymidine and incubated another 18 hours. The cells were harvested on to filtermats which were dried for one hours, placed in scintillation vials (with scintillation cocktail added) and their radioactivity measured in a scintillation counter to give a stimulation index.

Results and Discussion

The preliminary findings (reported on October 31 or June 30/89 progress reports) have now been finalized. Also, we now have the results of additional tests that were in-progress at those times.

(1) Feasibility of a Murine Model for U. diversum Infection

This study was in progress on June 30 but the results were overlooked in the October 31 report. Unfortunately, the Balb/c mice were very variable in their response to intravaginal inoculation of U. diversum (only two of the 10 control mice were culture-positive at the first sampling at 7 days post-inoculation and only three of the estrogen-inoculated, experimental group were positive). Because subsequent samplings gave reduced numbers of culture-positive mice it was concluded that a murine system would not be a useful model for extrapolation to cattle.

(2) Immune Response to U. diversum Vaccination

An initial study on the response of heifers to subcutaneous vaccinaton with killed U. diversum showed stimulation of specific IgG_1 and IgG_2 in serum and VM but no stimulation of IgM or IgA. Subsequent challenge resulted in marked increases in IgA antibody in VM of vaccinates and placebo vaccinates. Therefore, although subcutaneous vaccination stimulated IgG antibodies in serum and VM, it failed to stimulate IgA production in VM.

Because of these results the 2nd vaccination trial was designed to study the response to intravaginal and subcutaneous vaccination with adjuvated vaccines. Subcutaneous vaccination (Group III) elicited high Ig (immunoglobulin) G_1 and IgG_2 antibody responses in serum and VM, minimal IgM, but no detectable IgA responses. Vaccination with cell membranes in CFA (Group II) and with whole cells in CFA (Group I) by VM route, stimulated high IgG_1 and IgG_2 antibody levels in serum and in VM, and low IgA levels in VM. Vaccination with cell membranes alone by VM route (Group IV) stimulated minimal IgG_1 and IgG_2 levels in serum and in VM, but no detectable IgA levels. However, subsequent intravaginal challenge with ud, resulted in characteristic granular vulvitis in all vaccinated and control heifers. Challenge infection stimualted a markedly increased IgA antibody response in the VM as compared to response to vaccination. The challenge also produced a slight increase in IgG_1 and IgG_2 levels in serum and inVM of vaccinated heifers but only a slight primary IgG_1 and IgG_2 serum response in the controls.

The heifers vaccinated by the SQ (Group III) and the VM (Groups I and II) routes with antigen in CFA developed significantly greater skin reactivities than the controls and/or those vaccinated with antigen alone (no adjuvant, Group 4). Histological evidence of a delayed-type hypersensitivity reaction in skin was observed in skin biopsies from the animals vaccinated wuth ud in CFA. Further evidence of a cell-mediated immune response was provided by the lymphocyte transformation

or activiation test since peripheal blood lymphocytes, from the heifers vaccinated with ud antigens in CFA, showed significantly greater transformation in the presence of ud antigen.

Vaccination by the SQ route (Group III) stimulated ud-specific IgG_1 and IgG_2 antibodies (but not the other two Ig classes) in serum and in VM. Vaccination locally (VM route, Groups I and II) resulted in significantly increased levels of IgG_1 and IgG_2 in serum and VM but only stimulated a slight IgA response in VM as compared to the marked IgA response in VM following challenge. Furthermore, the vaccination stimulated the cell-mediated arm of the immune system.

In summary. although vaccination with ud in CFA by the VM or SQ routes significantly increased IgG_1 and IgG_2 (but not IgA) levels in serum and VM, the susceptibility of all groups of vaccinated heifers to subsequent, intravaginal exposure to U. diversum indicated that there was not a definitive correlation between the presence of these vaccination-induced Ig classes and protection of the reproductive tract against challenge with a very large number of organisms.

(3) Additional Studies

Some additional, preliminary studies (not included in original grant application) have been started in order to define the major protective antigens of U. diversum. This has involved, by polyacrylamide gel electrophoresis and western blotting protocols, the attempted reaction of proteins from intact ud or from cell-membrane ud preparations with sera from vaccinated and challenge-exposed heifers to see if specific antibody-stimulating proteins can be identified. To date, up to four protein antigens that react reasonably strongly with antibodies from the vaccinated/challenged heifers have been observed. Further studies are necessary to further characterize these proteins and to see if they are the protection-inducing antigens of Ureaplasma diversum.

(4) Conclusions and Impact

We achieved the basic objective of characterizing the immune response of heifers to genital infection with ureaplasmas and to subcutaneous/intravaginal vaccination. It appears that a specific antibody type IgA) in VM is associated with the slow clinical recovery/clearance of the organisms. However, the vaccination protocols did not effectively stimulate the production of IgA in the VM and may account for the lack of protection noted in the experiments. We also demonstrated that cellular immunity, mediated by lymphocytes, was stimulated to a much greater degree by vaccination and a subsequent challenge infection as compared to infection alone. However, the relevance of cellular immunity to clinical recovery/clearance of the ureaplasmas could not be determined by this study.

At this time it is apparent that vaccination, as done in this project, can not be instituted to control granular vaginitis. However, the new information obtained is important for a better understanding of this infection and does lead to suggestions for further study that could result in an effective U. diversum vaccine.

(5) Papers Prepared and Presentations

To date two presentations have been made from this work at the "Conference of Research Workers in Animal Disease" at Chicago in November 1988 and November 1989 entitled:

"Ureaplasma diversum Infections in Vaccinated/Unvaccinated Beef Heifers: Vaginal Lesions, Bacterial Shedding and Mucosal/Humoral Antibody Response (1988) - G.M. Luvira and J.R. Saunders.

"Continued Studies on Local/Humoral Immunity to Ureaplasma diversum in Vaccinated Heifers (1989) - G.M. Luvira and J.R. Saunders.

Dr. G. Luvira is now writing his PhD thesis from work done on this project and his thesis defense will take place in July or August 1990. It is expected that up to four papers will be published from this thesis.

(6) Personnel Involved

Technician - K. Ho, August 1, 1988 - March 31, 1989
(9 months - 100% time);
- B. Remenda, May 1, 1989 - August 31, 1989 .
(4 months - 25% time).

Graduate Student - G.M. Luvira (CIDA salaried) -
January 1, 1988 - March 31, 1990 (75% time).

Other - J.R. Saunders - January 1988 - March 31, 1990
(5% time).

EXPERIMENT #2.

UREAPLASMA VACCINATION & CHALLENGE PROTOCOL

20 CULTURE-NEGATIVE HEIFERS

GROUPS:	I	II	III	IV	V
VAC'N 1	S-CF/V	M-CF/V	M-CF/SC	M/V	CON/V
VAC'N 2	SAME ANTIGENS WITH INC FREUND'S AT DAY 28				

CHALLENGE AT DAY 49 WITH HOMOLOGOUS STRAIN

OBSERVE TO DAY 98: SERUM/MUCUS AB; CULTURE & (CMI)

S = sonicated whole cells. M = cell membrane.
CF = complete Freund's adjuvant. V = intravaginal.
SC = subcutaneous.

CLINICAL FINDINGS IN VACCINATES & CONTROLS ON INTRAVAGINAL CHALLENGE WITH UREAPLASMA

	GRANULAR VULVITIS	
GROUP	AT DAYS	SEVERITY
I (S/V)	4,4,4,4	MILD-2, SEVERE-2
II (M/V)	4,4,5,4	MI-1, MO-2, SE-1
III (M/SC)	4,4,4,4	MO-2, SE-2
IV (M/V)	5,4,4,5	MI-4
V (CON)	4,54,5	MI-2, MO-2

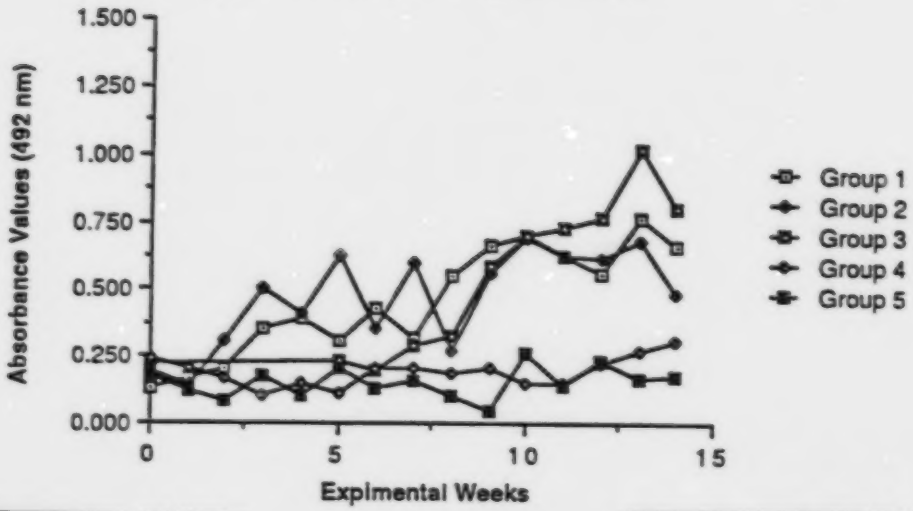
MO = moderate.

LYMPHOCYTE TRANSFORMATION POST-CHALLENGE

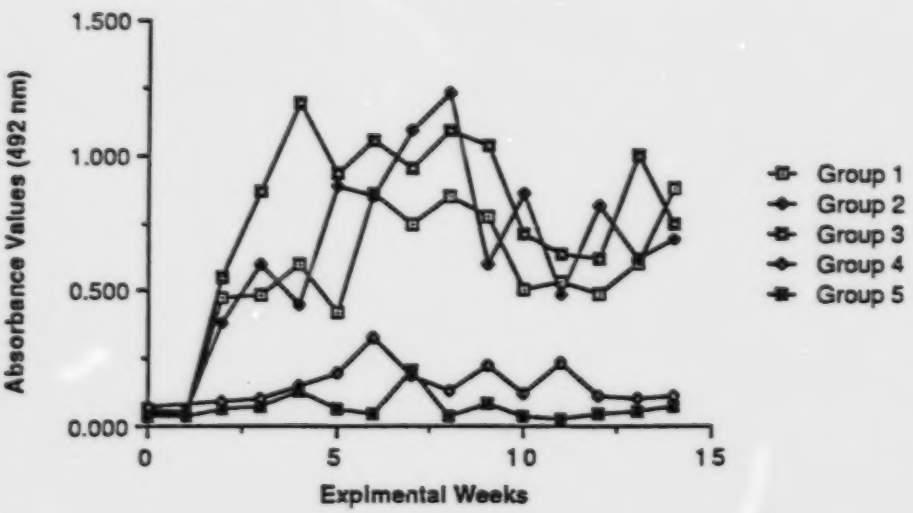
GROUP	STIMULATION INDEX
I	4.76 *
II	6.93 *
III	5.77 *
IV	1.40
V	1.15

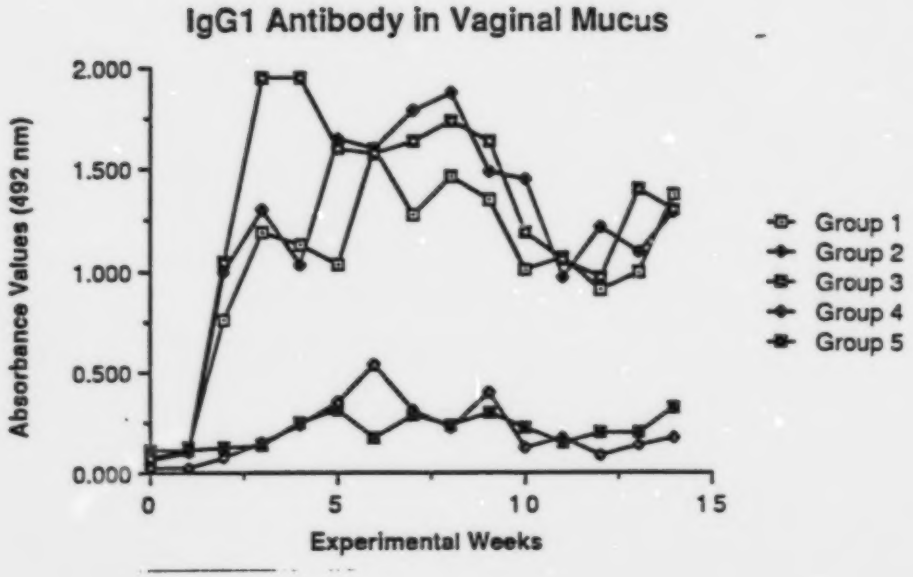
* Significant (compared to V and IV)

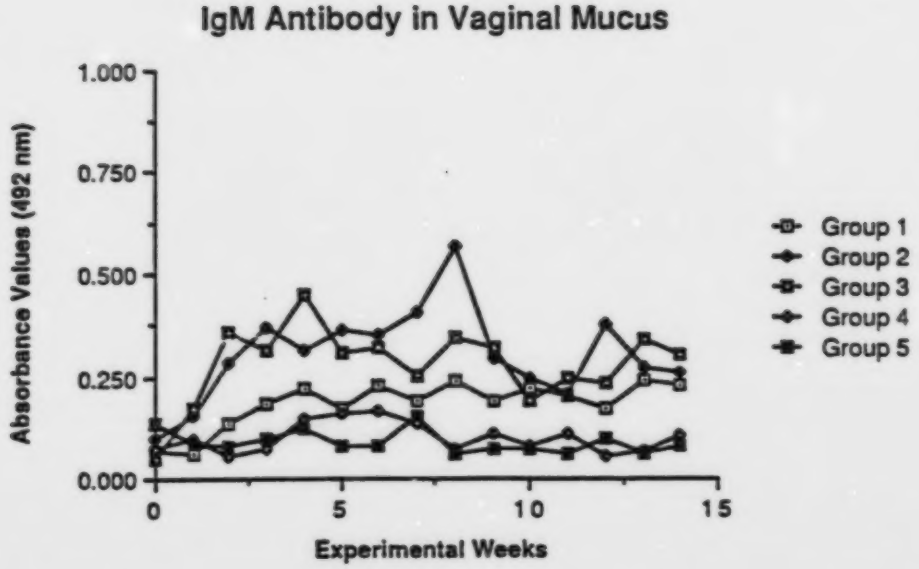
IgA Antibody in Vaginal Mucus



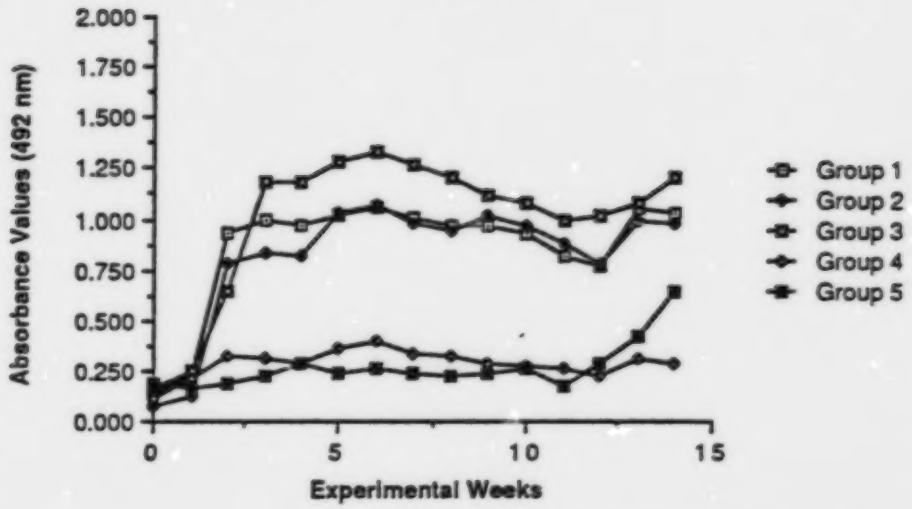
IgG2 Antibody in Vaginal Mucus



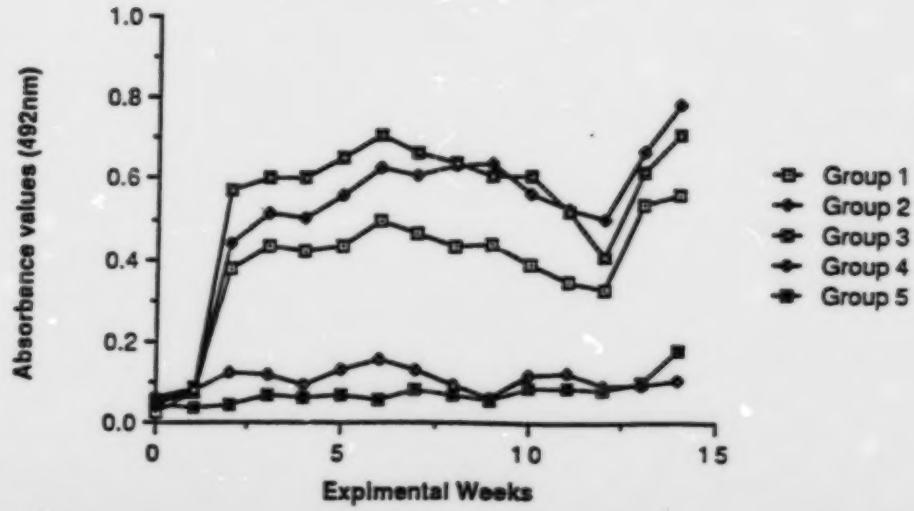

IgG1 Antibody in Vaginal Mucus
Absorbance Values (492 nm)
2.000
1.500
1.000
0.500
0.000
0
5
10
15
Experimental Weeks
Group 1
Group 2
Group 3
Group 4
Group 5

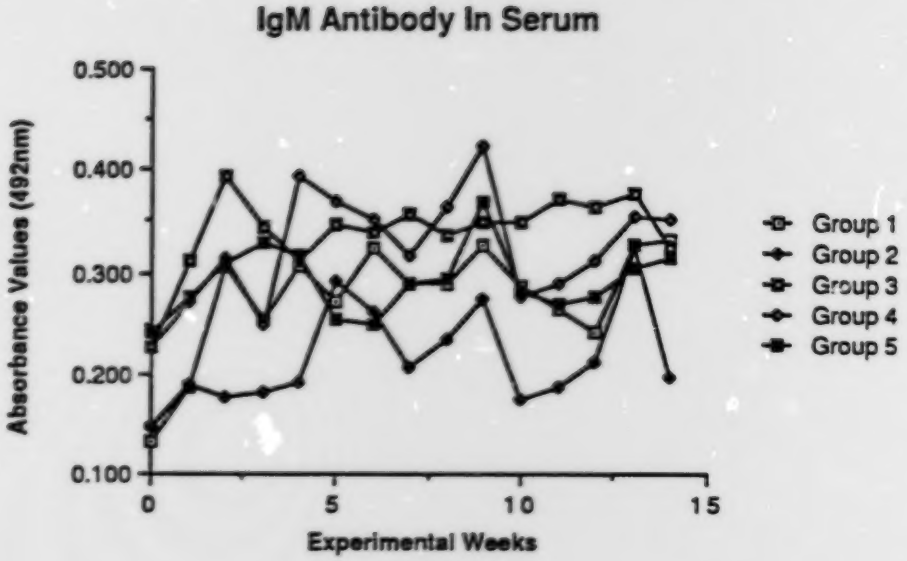

IgM Antibody in Vaginal Mucus
Absorbance Values (492 nm)
1.000
0.750
0.500
0.250
0.000
0
5
10
15
Experimental Weeks
Group 1
Group 2
Group 3
Group 4
Group 5

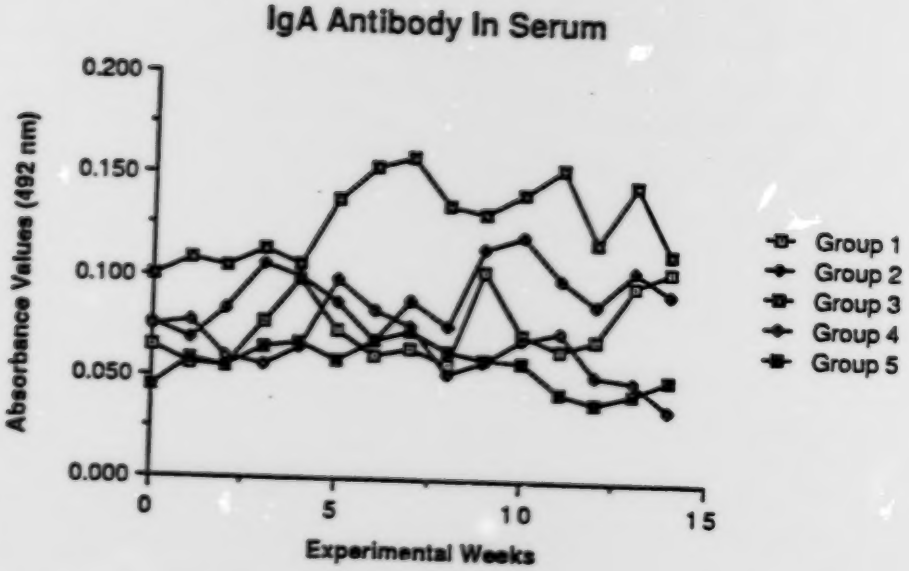
IgG1 Antibody in Serum



IgG2 Antibody in Serum




IgM Antibody In Serum
Absorbance Values (492nm)
0.500
0.400
0.300
0.200
0.100
0
5
10
15
Experimental Weeks
Group 1
Group 2
Group 3
Group 4
Group 5


IgA Antibody In Serum
Absorbance Values (492 nm)
0.200
0.150
0.100
0.050
0.000
0
5
10
15
Experimental Weeks
Group 1
Group 2
Group 3
Group 4
Group 5